

OCCURRENCE OF A THETA-LIKE ANTIGEN IN RATS*

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The theta antigen, described by Reif and Allen (1), provides a marker for thymus-derived lymphocytes in the mouse. The uses of this antigen system for studies in numerous fields, including immune system development, cellular cooperation, and the distribution of lymphocyte subpopulations, have recently been reviewed (2). Expression of the theta antigen is governed by a single autosomal locus having two alleles, θ^{AKR} and θ^{C3H} (3).

After immunization with θ -AKR antigen-bearing cells, spleen cells harvested from mice homozygous for θ^{C3H} have been shown to form plaques against thymus cells from several inbred rat strains as well as against those of mice carrying the θ^{AKR} allele (4). The present study was undertaken in order to clarify the relationship between this rat antigen system and the θ -system of mice.

Materials and Methods

Animals.—All inbred rat strains used in this study were obtained from Microbiological Associates, Inc., Bethesda, Md., with the exception of strain DA which was kindly provided by Dr. Joy Palm, The Wistar Institute, Philadelphia, Pa., and strain D which was originated in this laboratory by Dr. Ray D. Owen. AKR/J and C3H/HeJ mice were bought from the Jackson Laboratory, Bar Harbor, Maine, and AKR/Cum mice came from Cumberland View Farms, Clinton, Tenn.

Anti- θ Sera.—Antisera were produced according to the optimal schedule described by Reif and Allen (3). Blood was collected from the tail into heparinized tubes, and the plasma stored at -20°C until used.

Cytotoxicity Assay.—The cytotoxic test employed was similar to the one-step microcytotoxicity assay described by Amos (5), except for the substitution of Hanks' balanced salt solution (HBSS)¹ for barbital buffer in the thymocyte (TC) suspension, and of 0.3% trypan blue in HBSS for 0.25% trypan blue in saline. Test cells were preincubated with antiserum for 15 min at room temperature before complement addition. Test plates (No. 3034; Falcon Plastics, Div. B-D Laboratories, Inc., Los Angeles, Calif.) were then further incubated for 20 min at 37°C in a 6.3% CO_2 atmosphere. Exclusion of trypan blue dye was then used as the measure of test cell viability.

Complement.—Rabbit serum diluted 1/3 in HBSS (from which Ca^{++} and Mg^{++} had been omitted) was absorbed with purified agarose (Bio-Rad Laboratories, Richmond, Calif.) at 0°C for 30 min in the presence of 0.01 M ethylenediaminetetraacetic acid (EDTA) disodium

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¹ Abbreviations used in this paper: HBSS, Hanks' balanced salt solution; TC, thymocyte.

salt (6, 7). After filtration to remove the agarose beads, divalent cations were restored by the addition of CaCl_2 in an amount equimolar to the previously added EDTA. The pH of the absorbed serum was brought to approximately 7.4 with 0.1 M NaOH. 0.5-ml aliquots of absorbed sera were stored at -80°C until shortly before use.

Sera from rats of strains WF or F344 were used unabsorbed and were diluted 1/4 in HBSS before storage at -80°C .

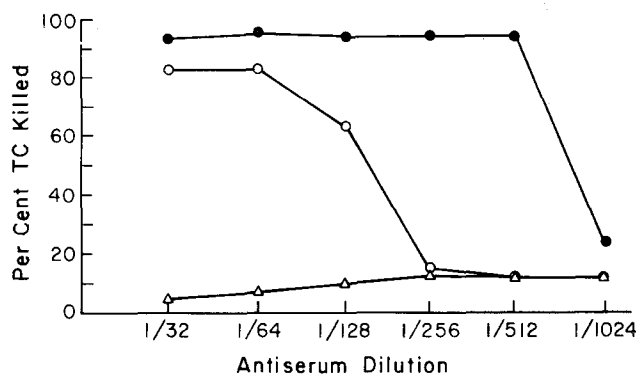


FIG. 1. Killing of AKR/J TC by C3H/HeJ anti-AKR/J serum. 100- μl aliquots of serum diluted to 1/32 in HBSS were absorbed with the following numbers of thymocytes: none (●—●), 1×10^7 DA rat TC (○—○), 1×10^7 AKR/J TC (Δ—Δ). Complement source: 1/12 agarose-absorbed rabbit serum.

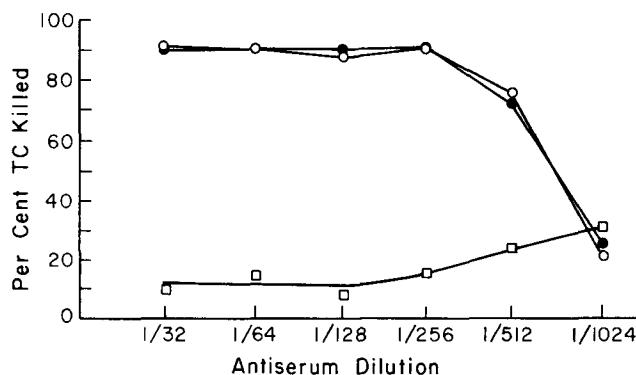


FIG. 2. Killing of C3H/HeJ TC by AKR/J anti-C3H/HeJ serum. Symbols and procedure same as in Fig. 1. Absorption with 1×10^7 C3H/HeJ TC (□—□).

RESULTS

Using AKR/J test cells and C3H/HeJ anti-AKR/J TC sera, rat thymocytes showed an intermediate absorptive capacity, relative to AKR/J TC (Fig. 1). However, using AKR/J anti-C3H/HeJ sera, negligible amounts of activity against C3H/HeJ cells were removed by rat thymocytes while C3H/HeJ TC showed strong absorption (Fig. 2). Control absorptions with TC of the serum donor's strain were also negative. Cytotoxic autoantibodies (8) were not observed in this system.

These absorption results were confirmed by the observation of direct killing of rat TC by mouse anti- θ serum in the presence of rat complement (Fig. 3). C3H/HeJ anti-AKR/J and AKR/Cum anti-AKR/J TC sera were both strongly cytotoxic for rat TC while C3H/HeJ and AKR/Cum normal sera were not. Also negative against rat TC were AKR/J anti-C3H/HeJ TC and AKR/J anti-AKR/Cum TC sera.

The complement source used to demonstrate the presence of θ -like antigen on rat thymocytes was of critical importance, as may be seen from Figs. 4 and 5. With rabbit complement, C3H/HeJ anti-AKR/J TC serum showed only a small differential killing effect. Using rat complement, however, this difference was striking.

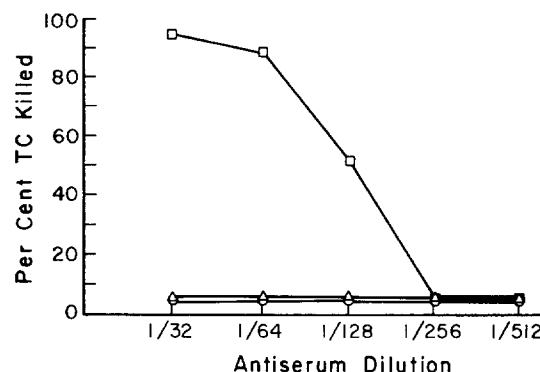


FIG. 3. Complement-mediated cytotoxicity of WF rat TC by anti- θ serum. Sera: C3H/HeJ anti-AKR/J TC (□—□), AKR/J anti-C3H/HeJ TC (Δ—Δ), C3H/HeJ normal serum (○—○). Complement source: 1/4 WF rat serum.

Preliminary tissue distribution experiments indicated that rat thymus and brain had the highest absorptive capacities for C3H/HeJ anti-AKR/J TC serum while rat liver, heart, testis, lung, skeletal muscle, erythrocytes, Peyer's patches, spleen, and peripheral white cells showed substantially less absorption. Based upon this observation, a developmental study of θ -like antigen in the rat nervous system was undertaken.

Rats were killed with chloroform and perfused with 0.85% NaCl. The brains were dissected out, weighed, and homogenized in saline so as to make suspensions containing 100 mg of tissue wet weight/ml of homogenate. All homogenates were made using Potter-Elvehjem type tissue grinders. Brain suspensions were stored at -20°C until used, at which time they were thawed and rehomogenized. Measured aliquots of homogenate were centrifuged at approximately 2150 g for 5 min and the supernatants discarded. 0.05-ml aliquots of AKR/Cum anti-AKR/J TC serum diluted $1/256$ in HBSS were then added. Absorptions were carried out for 30 min at room temperature with frequent stirring. The absorbed sera were centrifuged at 2150 g for 5 min, and

the supernatants tested for residual cytotoxic activity against AKR/J TC. Absorptive capacities were determined from von Krogh plots (Fig. 6 [9, 10]).

Fig. 7 shows that an approximately logarithmic increase in the rat brain's theta-absorptive capacity occurs between birth and approximately day 20, at

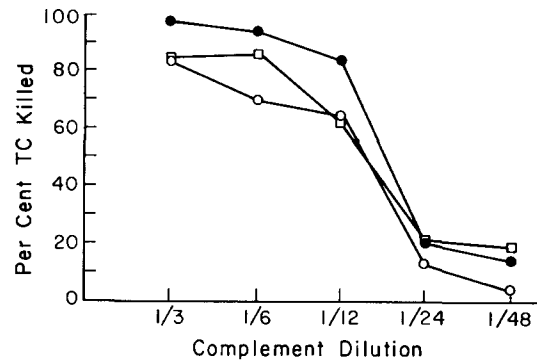


FIG. 4. Cytolysis of WF rat thymocytes by anti- θ serum and agarose-absorbed rabbit complement. Cells were incubated with or without anti- θ serum diluted 1/32 in HBSS. C3H/HeJ anti-AKR/J serum (●—●), AKR/J anti-C3H/HeJ serum (○—○), no serum (□—□)

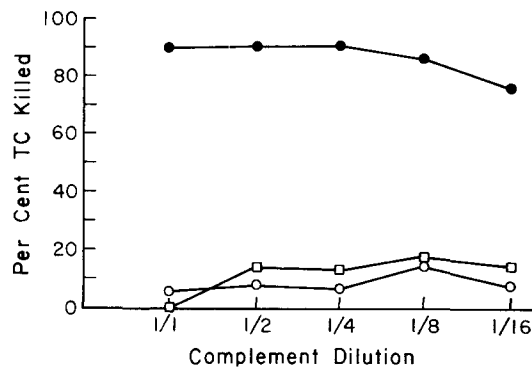


FIG. 5. Cytolysis of WF rat thymocytes by anti- θ serum and unabsorbed rat complement. Symbols same as for Fig. 4.

which point the slope of the curve rapidly declines. No significant difference in θ -absorptive capacity was observed between male and female rats.

To date, eight inbred rat strains have been tested for the presence of theta-like antigen. By both absorption and direct cytotoxic tests, thymocytes from rats of the ACI, BN, BUF, D, DA, F344, LEW, and WF strains all expressed antigenic determinants cross-reactive with the θ -AKR mouse antigen (Table I). No significant cross-reactivity with the θ -C3H antigen was observed. Wistar and Holtzman random-bred rats also gave similar results.

DISCUSSION

Quantitative absorption tests using mouse anti- θ sera and mouse thymocytes as target cells showed that rats of the inbred strains tested all expressed an antigen which was strongly cross-reactive with the θ -AKR, but not the θ -C3H, specificity of mice. The same pattern of cross-reactivity was seen when direct

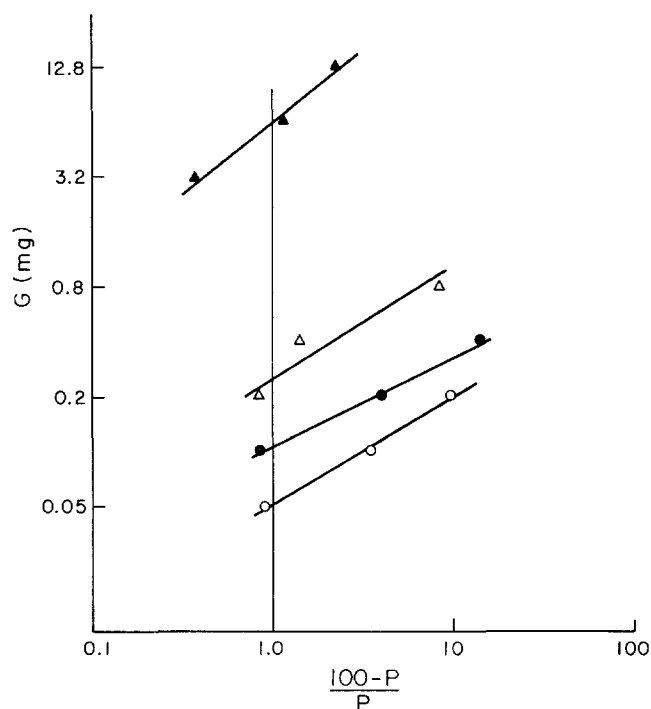


FIG. 6. Determination of the theta-absorptive capacities of WF rat brain homogenates. Postnatal age: 8 days (▲—▲), 18 days (△—△), 33 days (●—●), 271 days (○—○). Serum: AKR/Cum anti-AKR/J TC diluted 1/256 in HBSS. Complement source: 1/18 agarose-absorbed rabbit serum.

cytotoxic tests were performed using mouse anti- θ sera with rat thymocyte target cells.

The AKR/Cum mouse subline has been shown to be similar to the AKR/J subline with reference to several alloantigenic markers (Dr. E. A. Boyse, personal communication) while differing at the θ -locus (11). The cross-reactivity of AKR/Cum anti-AKR/J sera with rat antigens thus argues strongly that the specificity detected is indeed θ and not some other antigen.

As has been observed with rat lymphocytes (12) as well as with those of other species (13), the complement source used in cytolytic assays can be of critical importance. Although rat strains WF and F344 differ at three of the

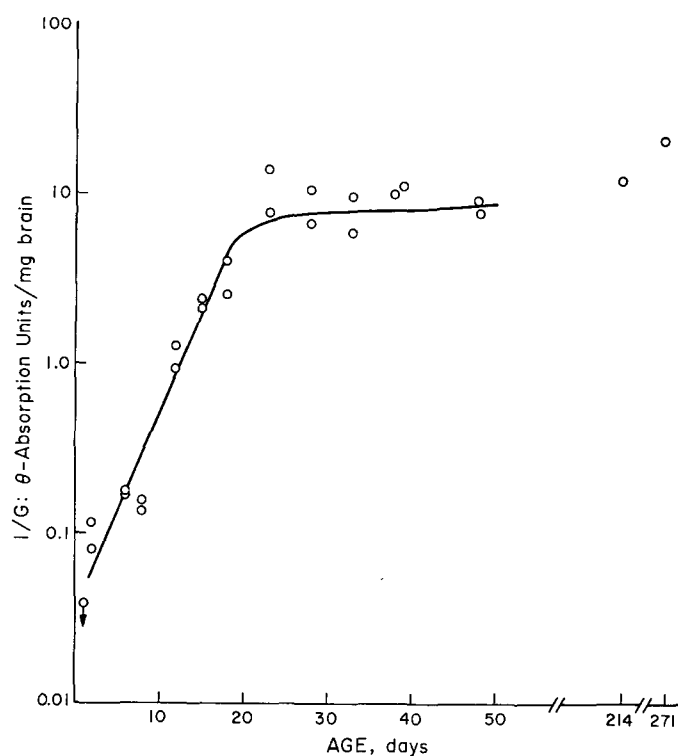


FIG. 7. Developmental appearance of θ -like specificity in WF rat brain. Absorptive capacities are expressed as θ -absorptive units = reciprocal of the number of milligrams of brain homogenate required to produce 50% inhibition of cytotoxicity. Serum: same as for Fig. 6. At 1 day, theta-absorptive capacity <0.04 .

TABLE I
Per Cent Rat TC Killed by Mouse Anti- θ Sera

| Rat strain | Serum | | | | | | | |
|------------|-------|----------------|----------------------------|----------------|----------------------------|-------------|----------------------------|----------------------------|
| | None* | AKR/ Cum NS | AKR/ Cum anti- AKR/J | C3H/ HeJ NS | C3H/ HeJ anti- AKR/J | AKR/J NS | AKR/ J anti- C3H/HeJ | AKR/ J anti- AKR/Cum |
| ACI | 18 | 12 | 58 | 11 | 77 | 17 | 19 | 15 |
| BN | 20 | 8 | 60 | 7 | 78 | 22 | 27 | 19 |
| BUF | 24 | 6 | 41 | 7 | 81 | 18 | 24 | 17 |
| F344 | 11 | 6 | 63 | 5 | 86 | 11 | 7 | 11 |
| LEW | 13 | 9 | 64 | 3 | 90 | 11 | 9 | 12 |
| WF | 14 | 10 | 77 | 10 | 81 | 16 | 16 | 14 |

All sera used at 1/32 dilution in HBSS. Note that this serum concentration does not necessarily represent a "cytotoxic plateau" for the respective sera, but merely gives a qualitative index of cytotoxic activity. Complement source: WF serum diluted 1/4 in HBSS.

* Preincubated with HBSS alone.

well-defined genetic loci for rat cellular antigens (14), their sera gave very similar results when used as complement sources for the immune cytolysis of thymocytes from six different strains of rats. The presence of rat natural antibodies (15) apparently does not pose a problem in this system, and rat serum provides a convenient source of complement for such testing.

In addition to exhibiting serological cross-reactivity with θ -AKR, the rat theta-like antigen also parallels the θ -antigen of the mouse in its tissue distribution and developmental kinetics (1, 16). Of particular interest is its occurrence at high concentration in brain tissue. In both species, neonatal brain expresses little or no antigen. During the first few weeks of postnatal life, however, expression of the antigen rapidly rises to the adult level.

The existence in rats of an antigenic system similar to the theta system of mice provides a potentially valuable resource for structural and functional studies of both the immune system and the nervous system. It is recommended that the new rat antigen be designated θ -R based upon its homology with the mouse θ -antigen. If allelic variants are found, they may be designated θ -R¹, θ -R², etc.

Other rat thymus (17-20) and brain (21, 22) antigens have been reported. Potworowski and Nairn (17, 18) used BALB/c mouse anti-Lister hooded rat TC microsome sera whereas Waksman and his collaborators (19, 20) prepared anti-rat TC sera in rabbits. Because BALB/c mice are homozygous for θ ^{C3H}, anti- θ -R-type antibodies might be expected to occur in sera raised against rat TC. The relationship between θ -R and the antigens described by Waksman are somewhat more difficult to assess. Activity paralleling that of anti- θ serum has been observed with rabbit anti-mouse TC and rabbit anti-mouse brain sera, but such reagents have not distinguished the genetic variants revealed by alloantisera (23-26).

SUMMARY

A rat antigen system parallel to the mouse theta system has been described. All rat strains tested expressed an antigen cross-reactive with the θ -AKR specificity of mice while none cross-reacted strongly with θ -C3H. The rat antigen may be demonstrated by either absorption or direct complement-mediated killing of rat thymocytes. Patterns of organ distribution and developmental appearance in the nervous system of rats also parallel those previously reported for theta in mice.

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